Determining the Race Structure of *Leptospheria maculans* in Western Canada

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Introduction

Blackleg disease of canola (*Brassica napus* L.), caused by *Leptospheria maculans* (Desmaz.) Ces. & De Not., is responsible for significant yield loss of *Brassica napus* L., oilseed rape and canola worldwide (Fig. 1). Resistant varieties and four-year rotations have controlled the disease very effectively in western Canada over the past 15 years. However, changes in the virulence of populations of *L. maculans* have been reported in western Canada recently (Chen and Fernando 2006, Kutcher et al. 2007).

Resistance in *B. napus* may be due to quantitative factors, or to specific resistance genes that interact with avirulence (*AvrLm*) genes of the pathogen in a gene-for-gene manner. Isolates have been classified into pathogenicity groups (PGs) based on reactions of the cultivars 'Quinta' and 'Glacier', which carry the specific resistance genes *Rlm1,3* and *Rlm2,3*, respectively. However, since there are presently 14 specific resistance genes reported, PGs do not provide characterization of isolates for avirulence genes other than those corresponding to *Rlm1*, *Rlm2* and *Rlm3*. By determining the frequency of avirulence alleles in populations of *L. maculans,* which correspond to resistance genes in the host, an understanding of the race structure of the pathogen can be obtained.

Fig 1 Blackleg infected canola plant in the field, cv 'Westar'

Objective

The objective of this project is to determine the race structure of *L. maculans* from isolates collected at 6 locations in western Canada. This information will be useful in the development of blackleg disease control strategies for canola in western Canada.

Materials and Methods

Westar canola was seeded in blocks as a trap crop at six locations in Western Canada in 2007 or 2008. Leaves and stems exhibiting blackleg symptoms were collected from each location during the growing season or after harvest. From this material isolations of *L. maculans* were made and isolates cultured from single pycnidia. Inoculum suspensions (1 x 107 spores mL-1) of each isolate were prepared and used to inoculate the differential cultivars: Westar - no R genes, Quinta

- *Rlm1,3*, Glacier - *Rlm2,3*, MT29 - *Rlm1,9*, Samourai - *Rlm2,9*, Quantum- *Rlm3*, Falcon - *Rlm4* and Darmor- *Rlm9*. Seven day old cotyledons of each of four plants of each differential were inoculated in two replicates using the standard cotyledon inoculation test. The plants were placed in a growth chamber at 22/16o C corresponding to a 16/8 hour photoperiod.

Disease symptoms, based on a scale of: 0 – no symptoms to 9 – severely diseased (Fig. 2, Newman 1980), were assessed at 14 days after inoculation. The data collected was analyzed to determine the frequency of each gene and the combination of genes that occurred at each location.

1 3 5 7 9

Figure 2. Cotyledon inoculation assessment scale modified from Newman, 1980.

Preliminary Results

- Characterization of isolates for avirulence genes corresponding to the five *B. napus* resistance genes examined, indicated that multiple races may occur within each pathogenicity group (Table 1).
- The percentage of isolates carrying *AvrLm1* was relatively low (<35%) at all locations except Plum Coulee, MB (73%) and Scott, SK (100%), although few isolates were examined at Scott (Fig. 3).
- The *AvrLm2* avirulence allele was carried by many isolates at each location (>70%), except Plum Coulee, MB (27%).
- *AvrLm3* was lowest at Plum Coulee, MB (22%) and Camrose, AB (33%), highest at the Saskatchewan locations (~85%) and intermediate at Brandon and Carberry, MB.
- *AvrLm4* was carried by between 36 and 63% of isolates at each location, except Brandon, where it was observed in 91% of isolates examined.
- *AvrLm9* was carried by only 16% of isolates from Plum Coulee, MB to as many as 94% at Scott, SK.
- These preliminary results indicate that there is considerable variation in the frequency of avirulence alleles of the pathogen among locations in western Canada.
- An understanding of the race structure of *L. maculans* in western Canada will provide the knowledge required to improve management of this disease through cultural measures, such as crop rotation; resistance gene management, such as cultivar rotation; and breeding, through the development of cultivars with improved resistance.

Table 1. Pathogenicity group (PGs) and race designation of isolates of *Leptosphaeria maculans* based on reactions on the differential set of varieties of *Brassica napus* carrying specific resistance genes. Some examples of the races obtained within each PG are listed.

PG	Race designation	Westar	Quinta *Rlm1,3*	Glacier *Rlm 2,3*	MT29 *Rlm 1,9*	Samourai *Rlm 2,9*	Quantum *Rlm 3*	Falcon *Rlm 4*	Darmor *Rlm 9*
PG2	Av(1)-(2)-3-9[2]	a	A[1]	A	A	A	A	a	A
PG2	Av1-2-4-9	a	A	A	A	A	a	A	A
PG2	Av1-2-4	a	A	A	A	A	a	A	a
PG2	Av1-2-9	a	A	A	A	A	a	a	A
PG2	Av2-3	a	A	A	a	A	A	a	a
PG3	Av1-9	a	A	a	A	A	a	a	A
PG3	Av1-4-9	a	A	a	A	a	a	A	A
PG3	Av1-4	a	A	a	A	a	a	A	a
PG4	Av4	a	a	a	a	a	a	A	a
PG4	Av9	a	a	a	A	A	a	a	A
PGT	Av2	a	a	A	a	A	a	a	a
PGT	Av2-4	a	a	A	a	A	a	A	a

[1] 'A' indicates an avirulent reaction of the host and pathogen; 'a' indicates a virulent reaction.
[2](1) or (2) indicates that *AvrLm1* and *AvrLm2* could not be confirmed due to the presence of confounding host R-genes.

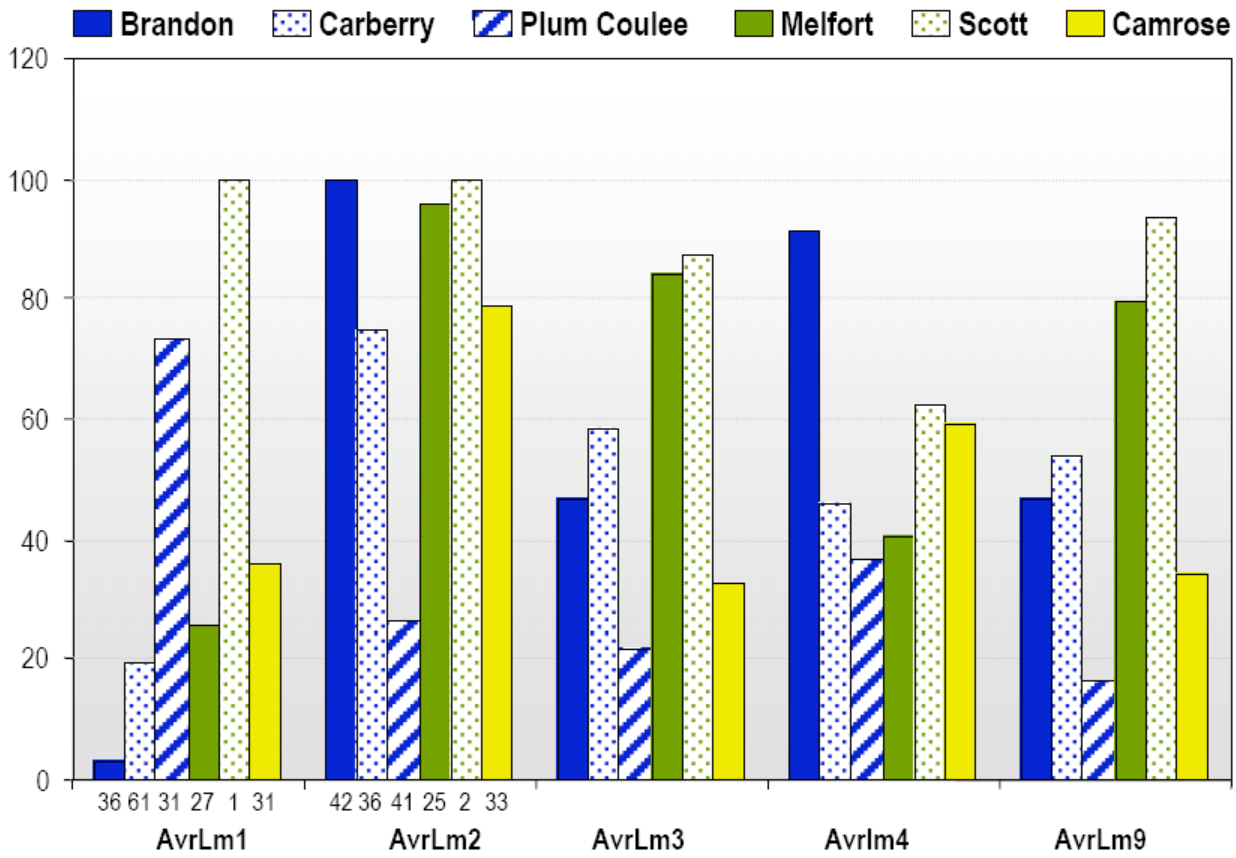

Figure 3. Percentage of *Leptosphaeria maculans* isolates carrying each avirulence gene. Data for *AvrLm3*, *AvrLm4* and *AvrLm9* are based on evaluation of 79, 87, 55, 93, 16 and 73 isolates at Brandon, Carberry, Plum Coulee, Melfort, Scott and Camrose, respectively. Data for *AvrLm1* and *AvrLm2* is based on a subset of isolates at each location, as indicated by the numbers below the columns on the graph. Alleles of these genes could not be determined for many isolates due to the presence of confounding host resistance genes.

References

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